

AMENDMENTS TO THE CLAIMS

The following listing of claims will replace all prior versions, and listings, of claims in this application.

Claim 1 (currently amended): A process for producing transglutaminase having an enzymatic activity comprising:

- (a) incubating a denatured transglutaminase in an acidic aqueous medium;
- (b) diluting the denatured transglutaminase in the acidic aqueous medium by about 5-fold to about 400-fold; and
- (c) adjusting the pH of said aqueous medium to a neutral pH by adding an alkali to said aqueous medium,

wherein said acidic aqueous medium in step (a) has a pH of from 3 to 5.

Claim 2 (original): The process as claimed in claim 1, wherein the aqueous medium further comprises a reducing agent.

Claim 3 (original): The process as claimed in claim 2, wherein the reducing agent is selected from the group consisting of dithiothreitol, 2-mercaptoethanol, and tris-(2-carboxyethyl)phosphine.

Claim 4 (original): The process as claimed in claim 1, wherein the denatured transglutaminase is obtained by a process comprising denaturing transglutaminase, which is expressed in a recombinant host cell, in the presence of a protein denaturant.

Claim 5 (original): The process as claimed in claim 4, wherein the protein denaturant is selected from the group consisting of urea, guanidine hydrochloride, and thiocyanate.

Claim 6 (original): The process as claimed in claim 4, wherein the transglutaminase concentration is from 10 to 100 mg/ml and the protein denaturant concentration is from 4 to 10 M.

Claim 7 (original): The process as claimed in claim 1, wherein the aqueous medium in step (a) further comprises a protein denaturant.

Claim 8 (original): The process as claimed in claim 7, wherein the protein denaturant is selected from the group consisting of urea, guanidine hydrochloride, and thiocyanate.

Claim 9 (previously presented): The process as claimed in claim 7, wherein the transglutaminase concentration is at least 40 mg/ml and the protein denaturant concentration is from 4 to 10 M.

Claims 10-11 (cancelled).

Claim 12. (currently amended) The process as claimed in claim 1, wherein ~~the~~ said acidic aqueous medium in step (a) ~~is of~~ has a pH of from 3.5 to 4.5.

Claim 13 (previously presented): The process as claimed in claim 1, wherein said denatured transglutaminase is diluted at least 5-fold.

Claim 14 (previously presented): The process as claimed in claim 1, wherein said denatured transglutaminase is diluted at least 10-fold.

Claim 15 (previously presented): The process as claimed in claim 1, wherein said denatured transglutaminase is diluted at least 50-fold.

Claim 16 (currently amended): The process as claimed in claim 1, wherein said incubation is performed at a temperature of not more than 15°C.

Claim 17 (currently amended): The process as claimed in claim 1, wherein said incubation is performed at a temperature of from 3 to 10°C.

Claim 18 (previously presented): The process as claimed in claim 1, wherein after said diluting in step (b) said denatured transglutaminase is at a concentration of not more than 10 mg/ml.

Claim 19 (original): The process as claimed in claim 1, wherein said neutral pH is from 5.8 to 8.5.

Claim 20 (original): The process as claimed in claim 1, wherein said neutral pH is from 6 to 7.

Claim 21 (previously presented): The process as claimed in claim 1, wherein in step (c), the aqueous medium further comprises an accelerator for forming a higher-order native-state transglutaminase structure having enzymatic activity.

Claim 22 (original): The process as claimed in claim 21, wherein the accelerator is selected from the group consisting of an inorganic salt, an organic salt, an amino acid salt, a polyol, an organic solvent, and a surfactant.

Claim 23 (previously presented): The process as claimed in claim 22, wherein the accelerator is an inorganic salt accelerator, which is selected from the group consisting of calcium chloride and strontium chloride.

Claim 24 (previously presented): The process as claimed in claim 23, wherein the inorganic salt accelerator concentration is from 0.01 to 10 mM.

Claim 25 (previously presented): The process as claimed in claim 22, wherein the accelerator is an organic salt accelerator, which is selected from the group consisting of sodium acetate and sodium propionate.

Claim 26 (previously presented): The process as claimed in claim 25, wherein the organic salt accelerator concentration is from 0.1 to 2 M.

Claim 27 (previously presented): The process as claimed in claim 22, wherein the accelerator is an amino acid salt accelerator and is arginine hydrochloride.

Claim 28 (previously presented): The process as claimed in claim 27, wherein the amino acid salt accelerator concentration is from 0.1 to 2 M.

Claim 29 (previously presented): The process as claimed in claim 22, wherein the accelerator is a polyol accelerator and is polyethylene glycol.

Claim 30 (previously presented): The process as claimed in claim 29, wherein the polyol accelerator concentration is from 1 to 10%.

Claim 31 (previously presented): The process as claimed in claim 22, wherein the accelerator is an organic solvent accelerator which is selected from the group consisting of DMSO and DMF.

Claim 32 (previously presented): The process as claimed in claim 31, wherein the organic solvent accelerator concentration is from 10 to 40%.

Claim 33 (previously presented): The process as claimed in claim 22, wherein the accelerator is a surfactant and is CHAPS.

Claim 34 (previously presented): The process as claimed in claim 33, wherein the surfactant concentration is from 1 to 50 mM.

Claim 35 (previously presented): The process as claimed in claim 1, further comprising:

(d) centrifugating the aqueous medium of (c).

Claim 36 (previously presented): An isolated transglutaminase obtained by the process of claim 1, which has a structure having a molecular ellipticity which is 30 to 70% of that of a native-state transglutaminase in a CD spectrum of a near ultraviolet region.

Claim 37 (previously presented): The process as claimed in claim 1, wherein step (c) further comprises incubating the aqueous medium for more than 1.5 hours subsequent to adjusting the pH to a neutral region.

Claim 38 (previously presented): A process for producing transglutaminase having an enzymatic activity, which comprises subjecting denatured transglutaminase to the following steps (a) and (b):

(a) a step for forming an intermediate transglutaminase structure; and

(b) a step for forming a higher-order native-state structure exhibiting substantially the same enzymatic activity as native transglutaminase.

Claims 39-40 (cancelled).

Claim 41 (original): The process as claimed in claim 38, wherein the denatured transglutaminase is obtained by a process comprising denaturing transglutaminase, which is expressed in a recombinant host cell, in the presence of a protein denaturant.

Claim 42 (original): The process as claimed in claim 41, wherein the protein denaturant is selected from the group consisting of urea, guanidine hydrochloride, and thiocyanate.

Claim 43 (original): The process as claimed in claim 41, wherein the transglutaminase concentration is from 10 to 100 mg/ml and the protein denaturant concentration is from 4 to 10 M.

Claims 44-71 (cancelled) .

Claim 72 (original): The process as claimed in claim 38, further comprising:

(c) a step for separating inactive enzyme(s) as aggregate(s) by centrifugation.

Claim 73 (previously presented): An isolated transglutaminase obtained by the process of claim 38, which has a structure having a molecular ellipticity which is 30 to 70% of that of a native-state transglutaminase in a CD spectrum of a near ultraviolet region.

Claim 74 (cancelled).

Claim 75 (original): A transglutaminase comprising the following properties (a) to (d):

(a) specific activity of 15 to 25 U/mg provided through measurement of transglutaminase activity by the hydroxamate method;

- (b) a molecular ellipticity which is 30 to 70% of that of the native state in a CD spectrum of a near ultraviolet region;
- (c) a molecular weight of 36,000 to 40,000 as measured by SDS-polyacrylamide gel electrophoresis; and
- (d) lower mobility than that of a native state in native-polyacrylamide gel electrophoresis with a His-Mes buffer system of pH 6.1.

Claim 76 (previously presented): A food comprising the transglutaminase of Claim 36.

Claim 77 (previously presented): The food of Claim 76, which is a jelly, yogurt, cheese or meat.

Claim 78 (previously presented): A toiletry comprising the transglutaminase of Claim 36.

Claim 79 (previously presented): A food comprising the transglutaminase of Claim 73.

Claim 80 (previously presented): The food of Claim 79, which is a jelly, yogurt, cheese or meat.

Claim 81 (previously presented): A toiletry comprising the transglutaminase of Claim 73.



Claim 82 (previously presented): A food comprising the transglutaminase of Claim 75.

Claim 83 (previously presented): The food of Claim 82, which is a jelly, yogurt, cheese or meat.

Claim 84 (previously presented): A toiletry comprising the transglutaminase of Claim 75.

Claim 85 (previously presented): In a method of producing a food comprising a transglutaminase, the improvement comprising producing the transglutaminase according to the process of Claim 1.

Claim 86 (previously presented): In a method of producing a food comprising a transglutaminase, the improvement comprising producing the transglutaminase according to the process of Claim 38.